Toxicopathic Liver Pathology in Flatfish from the Hylebos Waterway in Tacoma, Washington

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Introduction

Histopathological examination is widely recognized as a useful, rapid method for assessing injury in marine fishes due to the adverse acute and chronic effects of exposure to anthropogenic contaminants (Moore and Myers, 1994). Certain pathological conditions (lesions) in the liver of wild fish—including neoplasms, preneoplastic foci of cellular alteration (FCA), proliferative lesions, and specific or unique degenerative/necrotic lesions (SDN)—are clearly involved in the histogenesis of hepatic neoplasia and morphologically resemble lesions induced by experimental exposure of rodents (Maronpot et al., 1986) and fish (Schiewe et al., 1991; Hawkins et al., 1990; Hendricks et al., 1984) to a variety of anthropogenic chemical toxicants including carcinogens. These toxicopathic lesions (having an etiology related to exposure to toxic chemicals) also have been shown statistically to be positively associated with exposure to xenobiotic chemical contaminants in numerous field studies in various fish species (reviewed in Moore and Myers, 1994). Moreover, certain hepatic lesions in English sole have been associated with changes in serum chemistry parameters indicative of liver dysfunction (Casillas et al., 1985).

In the many studies done on English sole from Puget Sound and other sites on the Pacific Coast, the chemicals consistently found to most strongly influence the probability of occurrence of these liver lesions are polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and DDT and its derivatives. The one consistently significant biological risk factor for liver lesion occurrence in these studies is fish age, with risk of being affected by neoplasms and preneoplastic FCA rising incrementally with each year of age (Rhodes et al., 1987; Myers et al., 1991, 1994; O'Neill, this volume). Moreover, certain early biochemical responses to contaminant exposure (biomarkers) have been statistically associated with increased risk of toxicopathic liver lesion occurrence. These biomarkers include levels of metabolic conversion products of PAHs detectable as fluorescent aromatic compounds (FACs) in bile (Krahn et al., 1986; Myers et al., 1991, 1994, 1998); hepatic activities of the xenobiotic metabolizing enzyme cytochrome P4501A (CYP1A) inducible by PAHs and chlorinated hydrocarbons (CHs) (Myers et al., 1998; Collier and Varanasi, 1991), and hepatic levels of hydrophobic DNA adducts representing reactive intermediates of PAHs covalently bound to DNA bases (Myers et al., 1998; Stein et al., 1993). This DNA damage by carcinogens and subsequent generation of mutations is a necessary early step in the process of initiation of neoplasia (Stein et al., 1993). Therefore, certain liver lesions have clear utility as biomarkers of contaminant exposure and effects, and have become useful as indicators of environmental degradation in marine ecosystems.

English sole and rock sole are both relatively territorial benthic species that occupy their feeding grounds for the majority of the year, exhibit some degree of homing ability, and display only limited seasonal migrations related to reproductive activities (Day, 1976; Forrester, 1969; Holland, 1969). Therefore, they are useful sentinel species in studies assessing potential effects of chemical contaminants. Among the many field studies done on these species that have focused on histopathological examination and documentation of chemical exposure, several studies done in the late 1970s and early 1980s showed relatively high prevalences of toxicopathic hepatic lesions at multiple sites within Commencement Bay, including sites in the Hylebos Waterway (Malins et al., 1984; Becker et al., 1987). The data from these earlier studies, in part, formed the basis for the designation of Commencement Bay and several of its waterways, including the Hylebos Waterway, as priority Superfund sites in 1981. The existence of this historical data affords a unique opportunity to test the hypothesis that shore-based cleanup activities and

natural remediation processes in the Hylebos Waterway have significantly altered the prevalences of liver lesions known to be strongly associated with chronic exposure to chemical contaminants.

These previous studies were used as the basis for the present work. Although rock sole were sampled in the larger damage assessment study and show results similar to English sole (Collier et al., 1998), in the interest of brevity only the data for English sole will be presented here. The major objectives of this study were to (1) assess the extent of exposure to various chemical contaminants, and the magnitude of various early biochemical responses to this exposure (biliary FACs, hepatic CYP1A and hydrophobic DNA adducts) in English sole captured from sites in the Hylebos Waterway and a relatively uncontaminated reference site at Colvos Passage; (2) determine the prevalence of toxicopathic liver lesions in English sole from these same sites; (3) determine the relationships, if any, among various indices of chemical contaminant exposure and early responses to this exposure, and hepatic lesion occurrence in this species by various statistical methods; and (4) compare lesion prevalences and the magnitudes of early biochemical responses to contaminant exposure detected in the present study to available historical data from the Hylebos Waterway.

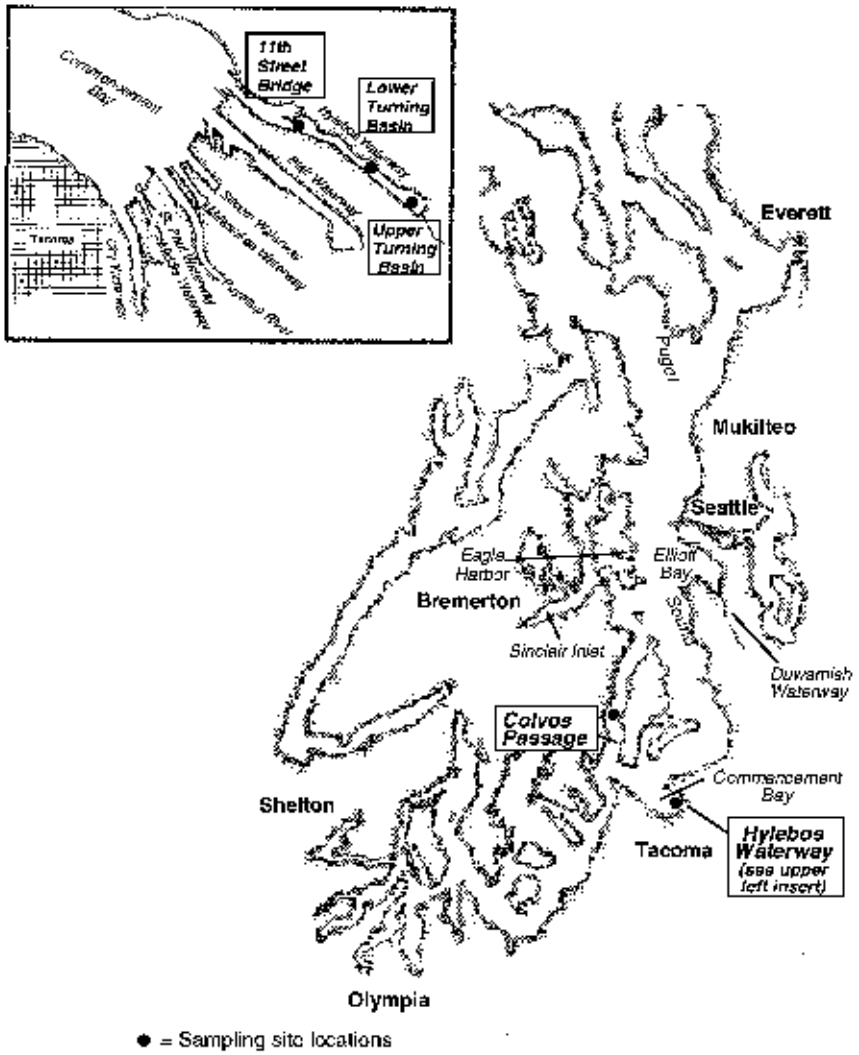

Figure 1. Sampling sites for flatfish toxicopathic injury study for the Hylebos Waterway.

Methods

Sample Collection and Analysis

Adult English sole were collected by otter trawl from sites in the Hylebos Waterway and a reference site in Colvos Passage (Figure 1), following standardized methods described in detail in Collier et al. (1998). Sampling was conducted in the Hylebos in July of 1994 and in Colvos Passage in September and October of 1994. Approximately 60 English sole at each site were collected from the Upper Turning Basin, lower Turning Basin, and 11th St. Bridge sites in the Hylebos Waterway, and from the Colvos Passage site. Otoliths were collected for age determination, and liver, kidney, and ovary were collected for histopathological examination; (data on kidney and ovarian lesions and other indicators of reproductive dysfunction are discussed in Collier et al. [1998] and Johnson et al. [this volume]). Liver, bile, and stomach contents were collected for measurement of chemical contaminant levels, and separate portions of liver from individual fish were also collected for measurement of CYP1A activity and DNA damage.

Methods for chemical and biological analyses (i.e., fish age determination, chemical analyses of liver tissue and stomach contents, measurement of biomarkers in bile and liver, and histopathological examination of fish tissues) are described in detail in Collier et al. (1998). Biliary FAC concentrations reported here and used in various correlative statistical analyses were adjusted for protein content of the bile sample, which corrects for variations in bile metabolite levels related to the feeding status of the sampled fish (Collier and Varanasi, 1991).

Statistical Methods

Details of statistical analyses performed are found in Collier et al. (1998). Briefly, analyses were performed: (1) to evaluate relationships among indices of contaminant exposure, early biochemical responses to exposure, and liver lesion occurrence; and (2) to compare data from English sole captured among three unique sites in the Hylebos Waterway, and the Hylebos Waterway as a whole, with data from the same species collected from the Colvos Passage reference site. Analysis of variance (ANOVA) (Zar, 1984) was used to identify significant intersite differences in chemical concentrations in liver and stomach contents, biliary FACs, and hepatic CYP1A and DNA adducts. Stepwise logistic regression (Breslow and Day, 1980) was used to identify significant relationships between potential risk factors and lesion occurrence. Two types of logistic regression analyses were conducted: (1) those examining the relative risk of disease, as estimated by the calculated odds ratio in individual fish, in relation to the variables of site of capture, age, biliary FACs levels, and hepatic DNA damage as adduct concentrations; and (2) analyses to assess the significance of the relationships between prevalences of lesions at the sampling sites and discrete risk factors, including levels of contaminants in liver and stomach contents, mean biliary FACs concentrations, mean CYP1A activities, and mean hepatic DNA adducts, while adjusting for mean fish age. In the latter analysis each sampling point for a species at a site was treated as an independent occurrence. These multivariate analyses permitted evaluation of contaminant-exposure/lesion-occurrence relationships, while simultaneously adjusting for fish age, a variable well known for its influence on risk of hepatic lesion occurrence (Rhodes et al., 1987; Myers et al., 1991, 1994, 1998; Moore and Myers, 1994; O'Neill et al., this volume).

For the first set of regression analyses above, odds ratios for particular lesion categories at a site were calculated and interpreted relative to the lesion occurrence at the Colvos Passage reference site. Increased probabilities of lesion occurrence were indicated by odds ratios greater than 1.000. Calculated odds ratios for age (in years) were interpreted for each additional year of age. For the second set of analyses, separate analyses were performed for each contaminant, contaminant class, biomarker of early response or risk factor discussed above, with results expressed as the significance of the association (*p* value). Because many of the contaminant exposure-related risk factors assessed in this analysis are known to be highly intercorrelated (Myers et al., 1994), it was not mathematically possible to include all risk factors into a single multivariate analysis. Consequently,

relationships between chemicals and chemical response risk factors and lesion prevalences were evaluated separately, while adjusting for potential effects of mean fish age at the sampling sites.

The contaminants, contaminant classes, and biomarkers of early response to contaminant exposure evaluated as risk factors for hepatic lesions were: high molecular weight aromatic compounds (HACs) and low molecular weight aromatic compounds (LACs) in stomach contents; PCBs, congener PCB 105, congener PCB 118, DDTs, hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), chlordanes (α + Γ), aldrin, dieldrin, heptachlor, and lindane in stomach contents and liver tissue; biliary FACs measured at benzo(a)pyrene wavelengths ($FACs_{BaP}$), at naphthalene wavelengths ($FACs_{NPH}$), and at phenanthrene wavelengths ($FACs_{PHN}$); and hepatic CYP1A and DNA adducts. A detailed description of these contaminants and classes is contained in Collier et al. (1998). These contaminants were measured and selected for analyses relating contaminant exposure to disease risk because they are found at high levels in sediments or fish from sites in embayments and estuaries located near major metropolitan centers (Myers et al., 1994), and because they represent broad classes of chemicals with documented toxic and/or carcinogenic potential in vertebrates (Klaasen et al., 1986) including fish (Meyers and Hendricks, 1982). The biomarkers of early response to contaminants were chosen because of their well known sensitivity to PAH exposure (biliary FACs, hepatic CYP1A, hepatic DNA adducts) and CH and pesticide exposure (CYP1A) in fish (Collier and Varanasi, 1991; Stein et al., 1993, 1994; Payne et al., 1987).

Pertinent data from male and female English sole form the "reproductive injury" portion of the broader damage assessment study and were incorporated into various statistical analyses in the present study; please refer to Collier et al. (1998) for information on sampling dates, number of English sole collected, and other relevant information.

Major Findings and Discussion

Objective 1: Determine Levels of Chemical Exposure in English Sole from the Hylebos Waterway

Adult English sole living in the Hylebos Waterway are clearly exposed to low and high molecular weight PAHs, PCBs, DDTs, HCB, and HCBD, regardless of whether the exposure is assessed by analyzing stomach contents (Figures 2–3), PAH metabolites in bile (Figure 4) or PCBs in liver (Figure 8) as an example of other CHs. Concentrations of these contaminants were generally 1–2 orders of magnitude higher in stomach contents of sole from Hylebos sites as compared to Colvos Passage reference fish. Among indices of exposure to PAHs, dietary exposure (stomach content LACs and HACs, Figure 2) was up to two orders of magnitude higher in sole from the Hylebos sites, and biliary FACs were 4–10 times higher at the Hylebos sites, with a clear pattern of increasing PAH exposure by both indices in sole from the sites further up the waterway (Figure 4). As a molecular dosimeter of exposure to genotoxic PAHs (Stein et al., 1992, 1994), DNA adduct levels in liver were also significantly higher in sole from Hylebos sites, showing progressively increasing levels in fish from sites further up the waterway (Figure 6).

Levels of dietary exposure to and hepatic bioaccumulation of PCBs (Figures 3, 5) and DDTs (data shown in Collier et al., 1998) were 15–25 times higher in sole from Hylebos sites compared to Colvos Passage reference fish, and like the PAHs, also showed a clear pattern of increasing exposure in English sole from the upper two sampling sites in the Hylebos Waterway. Exposure to HCB and HCBD, chemicals that are markers for the complex mixture of CHs in Hylebos Waterway sediments, was most pronounced in sole from the 11th St. Bridge site (data shown in Collier et al., 1998).

Levels of hepatic CYP1A induction, a biochemical response to organic contaminants such as PAHs and CHs, were significantly higher for English sole living in the Hylebos Waterway than those from the Colvos Passage reference site, regardless of where in the waterway they were captured (Figure 6).

Overall, there was a high degree of consistency for all exposure indices measured (except those for HCB and HCBD, and CYP1A in liver), demonstrating increased exposure and biochemical response to

exposure moving from the near the mouth to the head of the Hylebos Waterway. Although the data for rock sole are not discussed specifically in this paper, it is important to note this consistency was also evident in the close similarity in exposure levels among rock and English soles from the single common sampling site in the Hylebos (11th St. Bridge). The marked differences in many exposure parameters for fish captured among the three sites sampled in the Hylebos Waterway, the consistency among different measures of exposure to the same chemical class, and site-specific parallels in exposure data between the two target species (see Collier et al., 1998 for rock sole exposure data) clearly show that exposure closely reflects the site of capture within the Hylebos Waterway. These data lead us to conclude that there is fidelity in feeding areas and limited movement of fish among the Hylebos sites.

Objective 2: Determine Prevalences of Toxicopathic Liver Lesions in English Sole from the Hylebos Waterway

We found significantly increased relative risks for occurrence of several liver lesion categories that were associated with residence at sites within the Hylebos Waterway, while accounting for the important variable of fish age (Figures 7–8). Although low prevalences of certain lesion categories occurred in both species at the reference site, this is not a unique finding based on previous studies on these species in Puget Sound (Malins et al., 1984; Myers et al., 1991, 1994; Krahn et al., 1986; Becker et al., 1987; O'Neill et al., this volume). Significantly increased relative risks were shown for several lesion categories in English sole from sites in the Hylebos Waterway, relative to Colvos Passage (Figure 7). Estimated relative risks of 2.7–74 times that for comparably aged English sole from Colvos Passage were determined for SDN, proliferative lesions, and "any toxicopathic liver lesion," for example, at the three separate Hylebos sites. Relative risks for SDN were significantly and consistently higher at all three sites, with progressively higher relative risks as one moved up the waterway. In analyses combining all data from both the toxicopathic injury and reproductive injury studies (Figure 8), increased relative risks were shown for these same lesion categories, at values up to 26 times the baseline risk at Colvos Passage.

In prior studies of English sole from Puget Sound using this same statistical method, relative risks for hepatic lesion occurrence ranged from 2.7 (SDN, South Seattle Waterfront) to 8.7 (neoplasms, Upper Duwamish Waterway) when fish from Port Madison were used as the reference population (Rhodes et al., 1987). In studies incorporating data from the Puget Sound Ambient Monitoring Program (PSAMP, 1989–1993) and using a much larger database from multiple reference sites to establish a baseline (Myers et al., 1995), significantly increased estimated relative risks (RR_e) of lesion occurrence at a sampling site in Commencement Bay were shown for neoplasms (RR_e = 7.7), FCA (RR_e = 7.7) and SDN (RR_e = 11). Similar estimated relative risks occurred at the Elliott Bay site (range 2–20), and much higher ones (range 31–71) at the Duwamish Waterway site. If the lesion occurrence and age data in Hylebos English sole from the present study were to be analyzed and compared to the reference site data from either of these prior studies (where lesion prevalences were much lower than those shown in Colvos Passage English sole), it is highly probable that the estimated relative risks of lesion occurrence in English sole from the Hylebos sites in the present study would have approached or exceeded those shown for the Commencement Bay and Elliott Bay sites in that prior study. For example, prevalences in English sole from Commencement Bay in that study were ~5% for SDN, 12% for FCA, and 5% for neoplasms, and in Elliott Bay English sole were 10% for SDN, 11% for FCA, and 5% for neoplasms. Prevalences of the same lesion categories in English sole from the Hylebos sites in the current study were similar, and in some cases higher .

Objective 3: Determine Risk Factors Associated with Liver Lesion Occurrence in English Sole from the Hylebos Waterway

The results of logistic regression analyses assessing potential relationships between contaminant exposure and injury in the form of toxicopathic liver lesions (see Collier et al., 1998 for detailed data) were quite similar to those previously conducted on English sole (Myers et al., 1991, 1994, 1995; Rhodes

et al., 1987; O'Neill et al., this volume). Exposure to PAHs, PCBs (including mono-ortho substituted toxic coplanar congeners PCB 105 and PCB 118), and DDTs were the risk factors most commonly associated with hepatic lesion occurrence (see Collier et al., 1998 for details). However, as in those previous studies, because of the covariance of these contaminants it is not possible to quantify the proportional risk attributable to each of these chemical classes. Nonetheless, PAHs are toxicologically meaningful risk factors because of the experimentally demonstrated hepatotoxicity and carcinogenicity of these compounds and their prominent role as genotoxicants in the initiation of carcinogenesis. In contrast, the mode of action for PCBs and DDTs appears to be as nongenotoxic promoters of carcinogenesis by virtue of their cytotoxicity and subsequent stimulation of cell proliferation (reviewed in Myers et al., 1987, 1994; Moore and Myers, 1994). In addition, experimental exposures of fish to these CHs have resulted in the formation of liver lesions identical to those included in the SDN category, including megalocytic hepatosis and nuclear pleomorphism (reviewed in Myers et al., 1987, 1994; Moore and Myers, 1994). Neither HCB nor HCBD were strong risk factors for toxicopathic hepatic lesions in English sole in the current study, primarily because levels of dietary exposure and hepatic bioaccumulation of these compounds were comparatively low (relative to those at the 11th St. Bridge site) at other sites in the Hylebos displaying higher lesion prevalences.

Results of logistic regression analyses relating mean hepatic CYP1A activity levels to lesion prevalences for English sole also showed CYP1A induction as a significant risk factor for the most common hepatic lesion, SDN (see Collier et al., 1998 for detailed data). These results are consistent with other studies in English sole from Puget Sound, and the role of CYP1A in the metabolism and bioactivation of PAHs to their genotoxic and cytotoxic intermediates, as well as its inducibility by and metabolism of CHs (Collier and Varanasi, 1991).

Finally, lesion prevalences in English sole from Hylebos Waterway sites generally paralleled levels of DNA adducts in liver from the same fish and same sites. Highest mean levels of adducts were in English sole at the Upper Turning Basin site where hepatic lesion prevalences were also highest. Also, in logistic regression analyses examining the increased risk of lesion occurrence attributable to hepatic DNA damage, adduct levels were significant risk factors for FCA, SDN and "any toxicopathic hepatic lesion" (see Collier et al., 1998 for detailed data). These results parallel those of prior studies (Myers et al., 1995b, 1998) and provide further evidence linking measures of PAC exposure to hepatic lesion occurrence in benthic fish, especially for lesions occurring early in the histogenesis of hepatic neoplasia such as SDN and FCA (Myers et al., 1987; Moore and Myers, 1994). Levels of DNA adducts in livers of Hylebos Waterway English sole from the current study were similar to those determined by comparable quantitation methods in sole from Eagle Harbor in Puget Sound prior to capping of PAH-contaminated sediments (Myers et al., 1995b; Collier and Myers, 1997), and those estimated from previous 1988 data (Stein et al., 1992) in sole from the Duwamish Waterway. Both of these sites are also highly contaminated (primarily with PACs and/or PCBs) urban sites in Puget Sound exhibiting high prevalences of liver lesions in resident flatfish species.

Objective 4: Determine whether Appreciable Changes in Liver Lesion Prevalence and Values for Biochemical Measures of Early Response to Contaminant Exposure Have Occurred Since the Late 1970s

Drawing from the several studies done on English sole between 1979 and 1985, there is neither a substantial decrease in injury as overall toxicopathic liver lesion prevalence at the specific Hylebos sites, nor in the Hylebos Waterway as a whole (Figure 9). More importantly, prevalences of most of the liver lesions in English sole from the Hylebos Waterway continue to far exceed those found in sole from relatively uncontaminated reference sites in Puget Sound. Current concentrations of biliary $FACs_{BaP}$ in English sole are well within the range of values determined in 1987–88 (Figure 10), and in the case of the Upper Turning Basin, are much higher than historical values. Similarly, the mean DNA adduct levels and CYP1A activities in liver found in the current fish injury study are within or exceed the mean values determined in 1987–88 (Figures 11 and 12).

Overall, the data do not provide evidence that natural or engineered remediation processes over the last 10–15 years have resulted in a substantial improvement in the extent of chemical exposure and associated liver injury to English sole in the Hylebos Waterway.

Figures

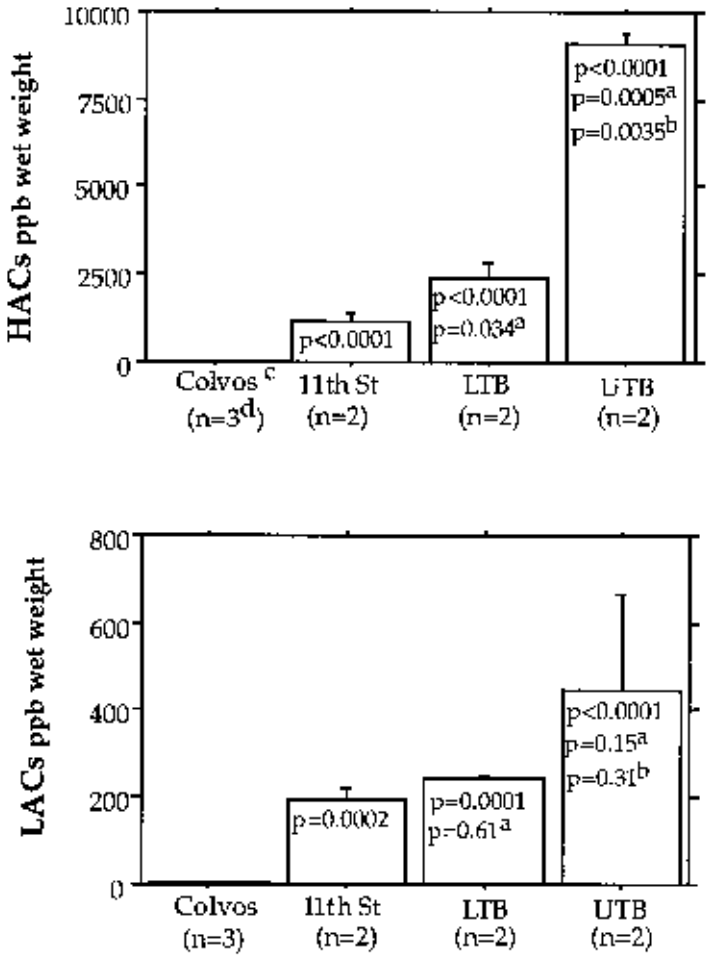

Figure 2. Concentrations of aromatic hydrocarbons in stomach contents of English sole from the Hylebos Waterway in Commencement Bay and from Colvos Passage, Washington. Values are mean concentrations ± one standard error (SE). The *p* (or probability) value shown is for the statistical comparison between Hylebos Waterway sites and the Colvos Passage reference site, unless otherwise noted. Mean concentrations and statistical comparisons are derived from an analysis of variance (ANOVA).

[a]*p* value for the upper Turning Basin or lower Turning Basin compared to the 11th Street Bridge site.

[b]*p* value for the comparison between the upper Turning Basin and lower Turning Basin sites.

[c]abbreviations: Colvos = Colvos Passage; 11th St = 11th Street Bridge site in Hylebos Waterway; LTB = lower Turning Basin site in Hylebos Waterway; UTB = upper Turning Basin site in Hylebos Waterway; HACs = high molecular weight aromatic compounds; LACs = low molecular weight aromatic compounds. Analytes included in HACs and LACs are listed in Appendix 2 of Collier et al. (1998).

[d]the number of analyses conducted (n) is indicated beneath each site name.

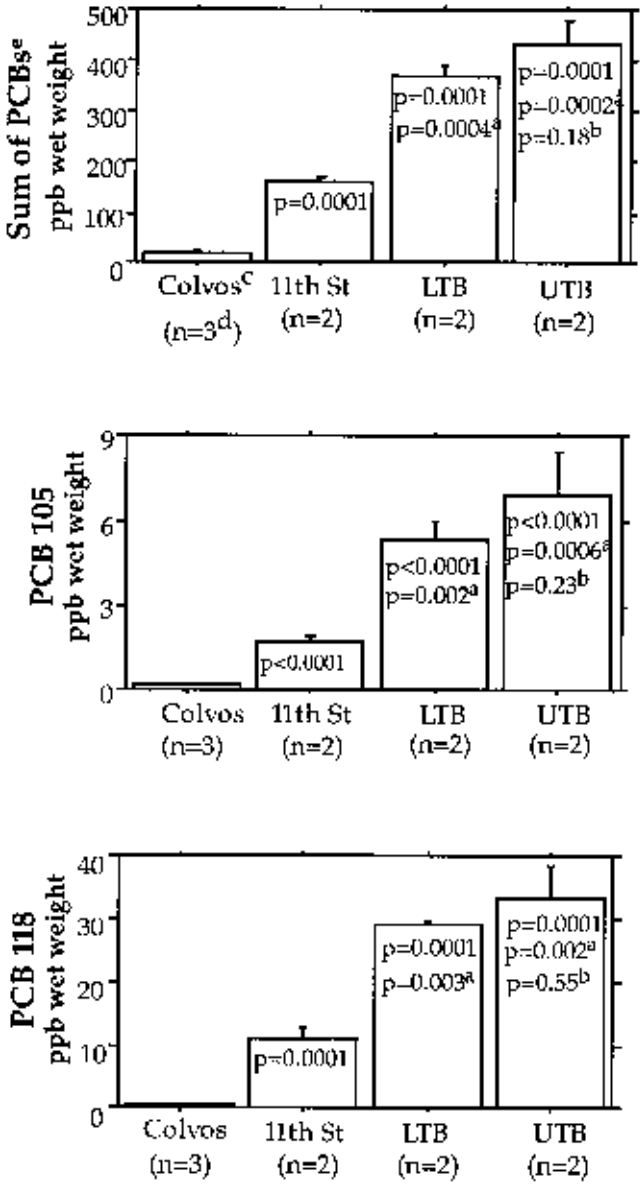

Figure 3. Polychlorinated biphenyls (PCBs) in English sole stomach contents sampled from the Hylebos Waterway in Commencement Bay and from Colvos Passage, Washington. Values are mean concentrations ±SE. The *p* value shown is for the statistical comparison between Hylebos Waterway sites and the Colvos Passage reference site, unless otherwise noted.

[a]*p* value for the UTB or LTB compared to the 11th Street Bridge site.

[b]*p* value for the comparison between the UTB and LTB sites.

[c]site abbreviations: Colvos = Colvos Passage; 11th St = 11th Street Bridge site in Hylebos Waterway; LTB = lower Turning Basin site in Hylebos Waterway; UTB = upper Turning Basin site in Hylebos Waterway.

[d]the number of analyses conducted is indicated beneath each site name.

[e]analytes included in the sum of PCBs are listed in Collier et al. (1998).

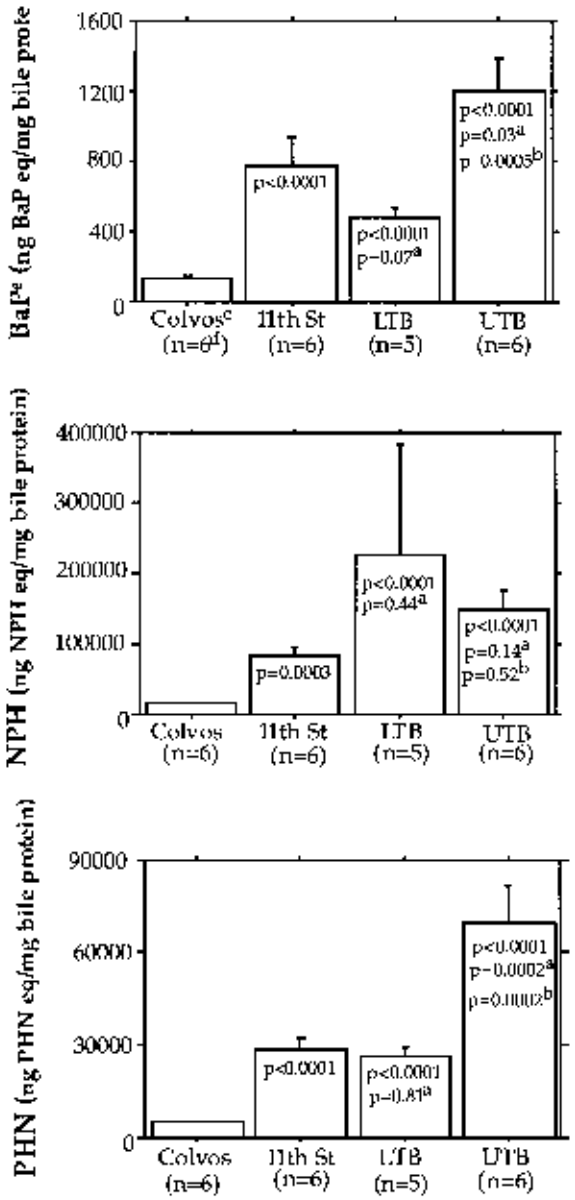

Figure 4. Concentrations of fluorescent aromatic compounds (FACs) in bile of English sole from the Hylebos Waterway in Commencement Bay and from Colvos Passage, Washington. Values are mean concentrations +SE. The *p* value shown is for the statistical comparison between Hylebos Waterway sites and the Colvos Passage reference site, unless otherwise noted.

[a]*p* value for the UTB or LTB compared to the 11th Street Bridge site.

[b]*p* value for the comparison between the UTB and LTB sites.

[c]site abbreviations: Colvos = Colvos Passage; 11th St = 11th Street Bridge site in Hylebos Waterway; LTB = lower Turning Basin site in Hylebos Waterway; UTB = upper Turning Basin site in Hylebos Waterway.

[d]the number of analyses conducted is indicated beneath each site name.

[e]BaP = mean values for benzo[a]pyrene wavelength equivalents (sum of high molecular weight FACs); NPH =mean values for naphthalene wavelength equivalents (sum of low molecular weight FACs); PHN = mean values for phenanthrene wavelength equivalents (sum of mid-level molecular weight FACs). Biliary FACs have been adjusted for protein concentration.

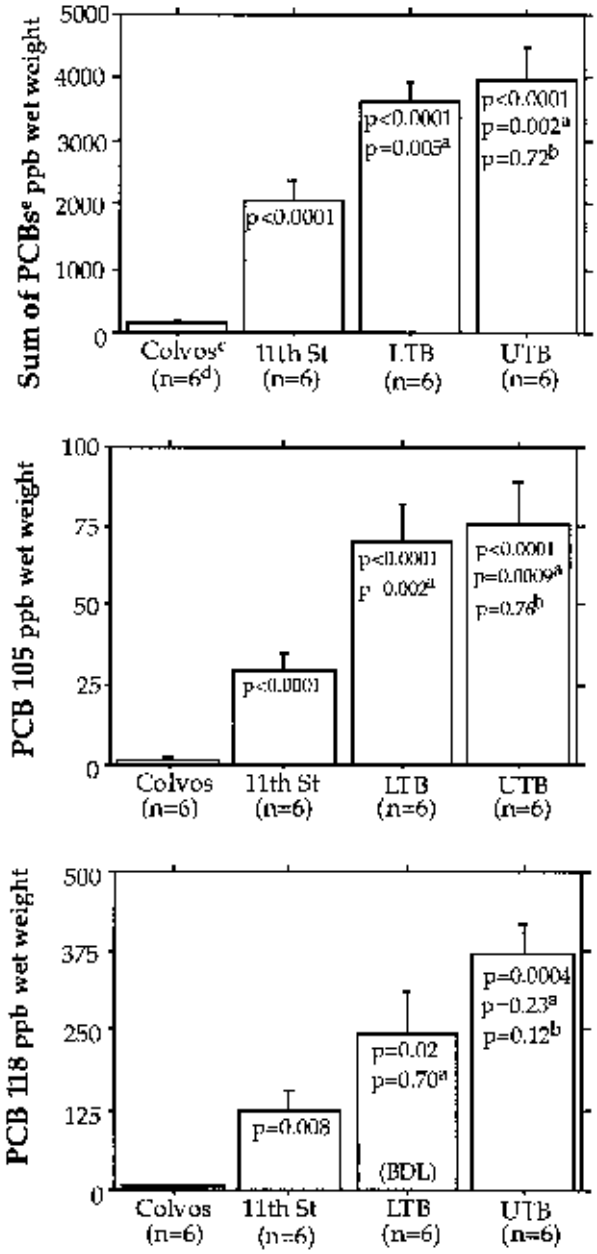

Figure 5. Polychlorinated biphenyls (PCBs) in English sole liver from the Hylebos Waterway in Commencement Bay and from Colvos Passage, Washington. Values are mean concentrations ±SE. The *p* value shown is for the statistical comparison between Hylebos Waterway sites and the Colvos Passage reference site, unless otherwise noted.

[a]*p* value for the UTB or LTB compared to the 11th Street Bridge site.

[b]*p* value for the comparison between the UTB and LTB sites.

[c]site abbreviations: Colvos = Colvos Passage; 11th St = 11th Street Bridge site in Hylebos Waterway; LTB = lower Turning Basin site in Hylebos Waterway; UTB = upper Turning Basin site in Hylebos Waterway.

[d]the number of analyses conducted is indicated beneath each site name.

[e]analytes included in the sum of PCBs are listed in Collier et al. (1998).

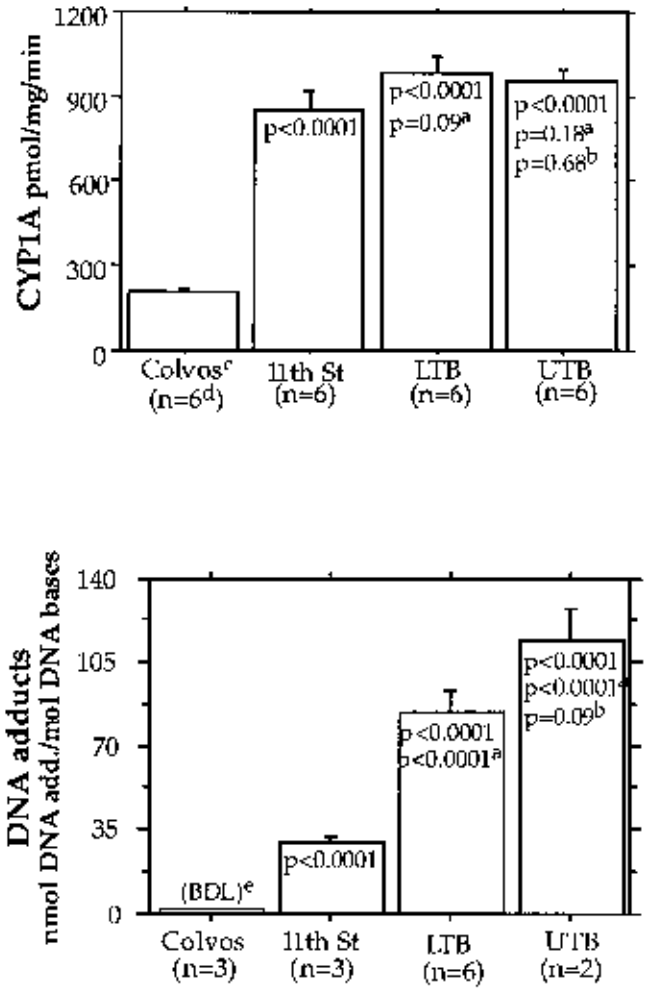

Figure 6. Cytochrome p4501A and DNA adducts in English sole liver from the Hylebos Waterway in Commencement Bay and from Colvos Passage, Washington. Values are mean concentrations ±SE. The *p* value shown is for the statistical comparison between Hylebos Waterway sites and the Colvos Passage reference site, unless otherwise noted.

[a]*p* value for the UTB or LTB compared to the 11th Street Bridge site.

[b]*p* value for the comparison between the UTB and LTB sites.

[c]site abbreviations: Colvos = Colvos Passage; 11th St = 11th Street Bridge site in Hylebos Waterway; lTB = lower Turning Basin site in Hylebos Waterway; UTB = upper Turning Basin site in Hylebos Waterway.

[d]the number of analyses conducted is indicated beneath each site name.

[e]BDL = some or all of the samples in this group had concentrations that were below detection limits. Detection limits vary depending on the sample size; detection limits for specific samples are listed in the case narrative. Samples with values below detection limits were treated as if the concentration was 50% of the detection limit.

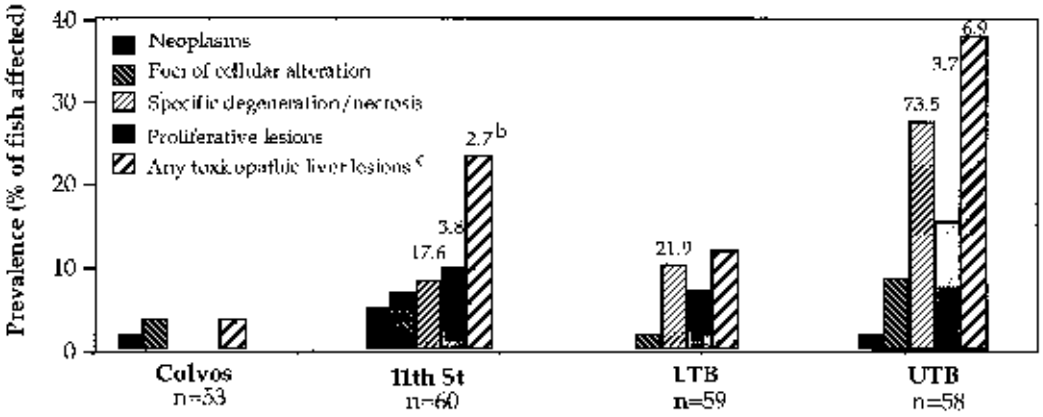

Figure 7. Prevalences of toxicopathic hepatic lesions in English sole from unique sites in the Hylebos Waterway in Commencement Bay and from Colvos Passage, Washington.

[a]site abbreviations: Colvos = Colvos Passage (reference site); 11th St = 11th Street Bridge site in Hylebos Waterway; LTB = lower Turning Basin site in Hylebos Waterway; UTB = upper Turning Basin in Hylebos Waterway.

[b] numbers above lesion bars indicate the significantly higher (p<0.05) estimated relative risks for that lesion category, as compared to fish from Colvos Passage (data combined from both studies, n = 195), by logistic regression while accounting for effects of fish age on probability of lesion occurrence.

[c]the "any toxicopathic liver lesion" category includes fish having one or more toxicopathic lesion types including: neoplasms, foci of cellular alteration, specific degenerative/necrotic lesions, and proliferative lesions.

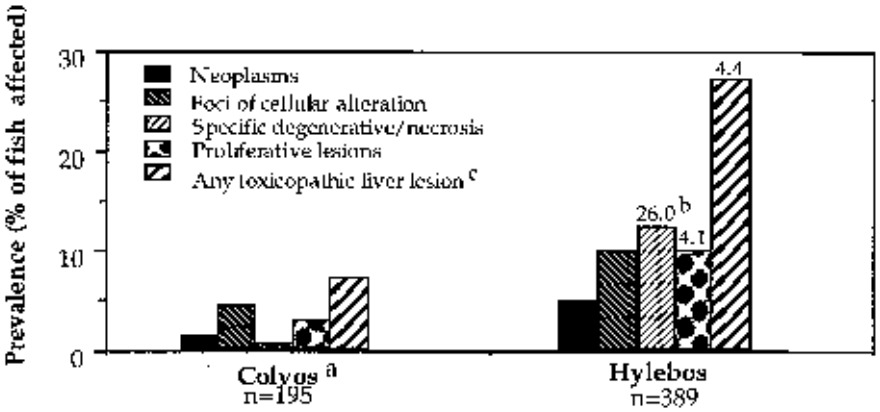

Figure 8. Prevalences of toxicopathic hepatic lesions in male and female English sole sampled during the combined toxicopathic injury and reproductive injury portions of the 1994–95 fish injury studies of the Hylebos Waterway in Commencement Bay and from Colvos Passage, Washington.

[a]site abbreviations: Colvos = Colvos Passage (reference site); Hylebos = Hylebos Waterway.

[b]numbers above lesion bars indicate the significantly higher (p<0.05) estimated relative risks for that lesion category, as compared to fish from Colvos Passage (data combined from both studies, n = 195), by logistic regression while accounting for effects of fish age on probability of lesion occurrence.

[c]the "any toxicopathic liver lesion" category includes fish having one or more toxicopathic lesion types including neoplasms, foci of cellular alteration, specific degenerative/necrotic lesions, and proliferative lesions.

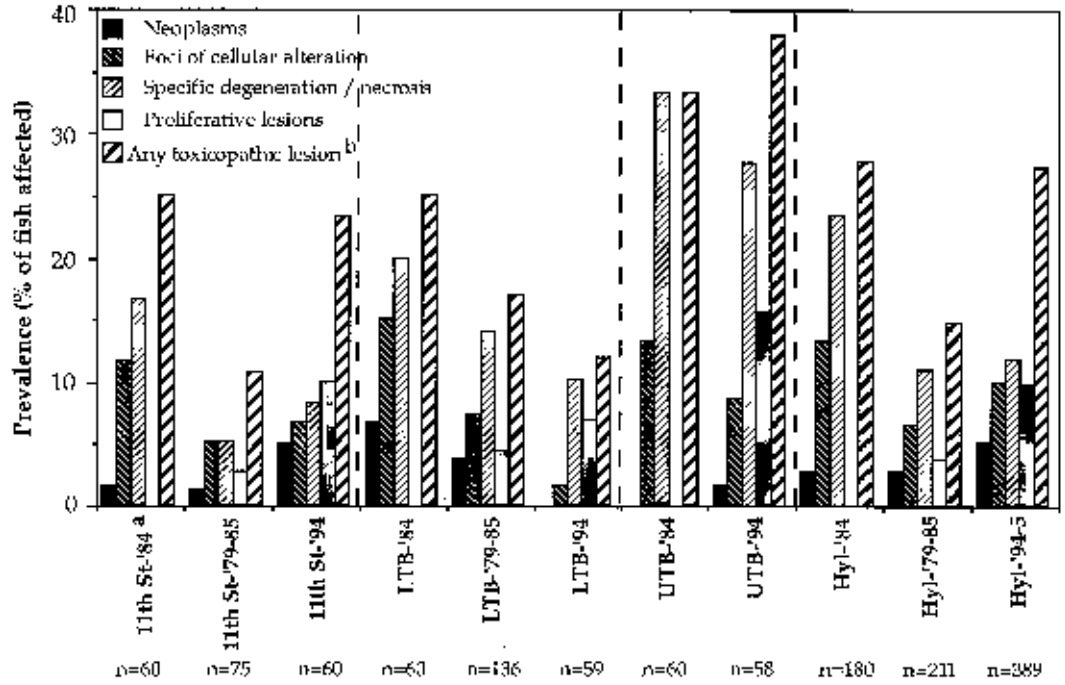

Figure 9. Prevalences of toxicopathic liver lesions in English sole from the Hylebos Waterway in Commencement Bay, Washington, compared to historical data from the Hylebos Waterway.

[a]site abbreviations: 11th St -'94 = 11th St. Bridge site in Hylebos Waterway, 1994; LTB-'94 = lower Turning Basin site in Hylebos Waterway, 1994; UTB-'94 = upper Turning Basin in Hylebos Waterway, 1994; Hyl-'94–5 = combined data from any Hylebos Waterway site, 1994–95; Hyl-'79–85 = combined data from multiple studies done in Hylebos Waterway by NWFSC; 11th St-'84, LTB-'84, UTB-'84 = data from Becker et al. (1987) at the same sites as above.

[b]the "any toxicopathic liver lesion" category includes fish having one or more toxicopathic lesion types including neoplasms, foci of cellular alteration, specific degenerative/necrotic lesions, and proliferative lesions.

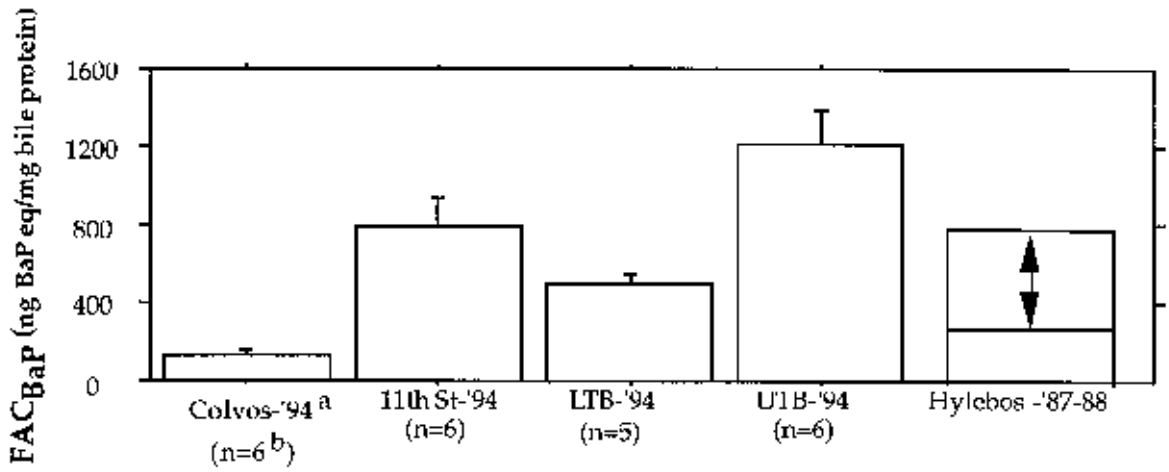

Figure 10. Concentrations of fluorescent aromatic compounds (FACsBaP) in bile of English sole from the Hylebos Waterway in Commencement Bay, and from Colvos Passage, Washington; comparisons with historical data from the Hylebos Waterway. Error bars indicate ± one standard error.

[a]site abbreviations: Colvos-'94 = Colvos Passage, 1994; 11th St-'94 = 11th Street Bridge site in Hylebos Waterway, 1994; LTB-'94 = lower Turning Basin site in Hylebos Waterway, 1994; UTB-'94 = upper Turning Basin site in Hylebos Waterway, 1994; Hylebos-'87–88 = range (indicated by arrows) of mean values determined in 1987–88 (Stein et al., 1992; Myers et al., 1998).

[b]the number of composite analyses conducted is indicated beneath each site name.

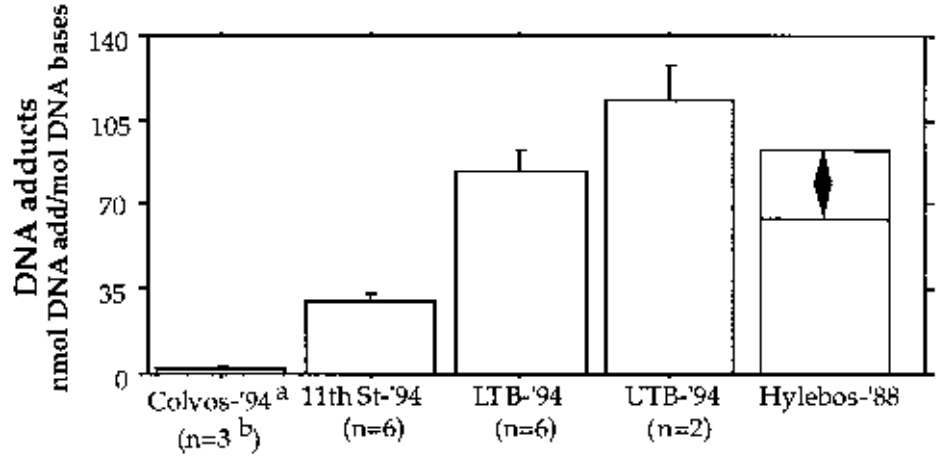

Figure 11. Concentrations of DNA adducts in liver of English sole from the Hylebos Waterway in Commencement Bay and from Colvos Passage, Washington; comparisons with historical data from the Hylebos Waterway. Error bars indicate ± one standard error.

[a]site abbreviations: Colvos-'94 = Colvos Passage, 1994; 11th St-'94 = 11th Street Bridge site in Hylebos Waterway, 1994; LTB-'94 = lower Turning Basin site in Hylebos Waterway, 1994; UTB-'94 = upper Turning Basin site in Hylebos Waterway, 1994; Hylebos-'88 and Hylebos Entrance-'88 = range (indicated by arrows) of values adjusted from previous data (May, 1988; Stein et al., 1992) based on changes in methods for quantifying DNA adduct concentrations.

[b]the number of composite analyses conducted is indicated beneath each site name.

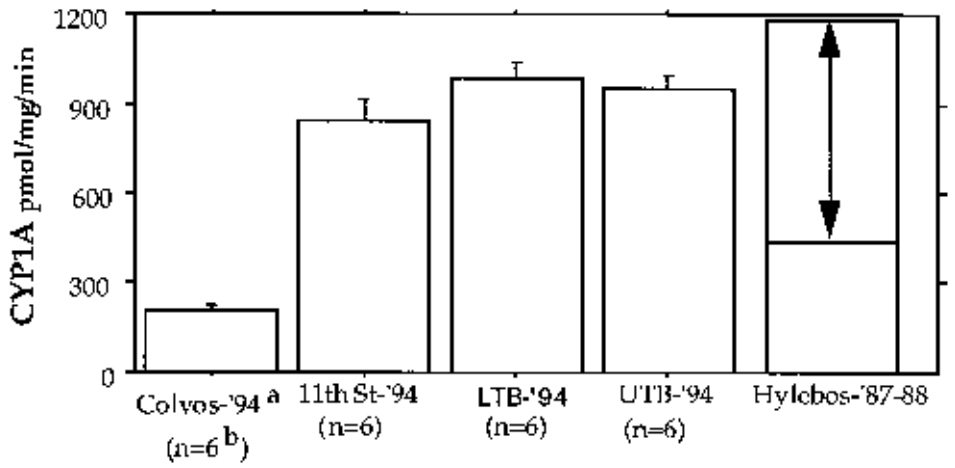

Figure 12. Activities of cytochrome p4501A (CYP1A) in liver of English sole from the Hylebos Waterway in Commencement Bay and in Colvos Passage, Washington; comparisons with historical data from the Hylebos Waterway. Error bars indicate ± one standard error.

[a]site abbreviations: Colvos-'94 = Colvos Passage, 1994; 11th St-'94 = 11th Street Bridge site in Hylebos Waterway, 1994; LTB-'94 = lower Turning Basin site in Hylebos Waterway, 1994; UTB-'94 = upper Turning Basin site in Hylebos Waterway, 1994; Hylebos-'87–88 = range (arrows) of mean values determined in 1987–88 (Stein et al., 1992; Myers et al., 1998). Error bars indicate ± one standard error.

[b]the number of composite analyses conducted is indicated beneath each site name.

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